

FORM PTO-1390
(REV 10-95)

U.S. DEPARTMENT OF COMMERCE AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

BHV-317.01

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

09/486516

INTERNATIONAL APPLICATION NO.
PCT/AT98/00202INTERNATIONAL FILING DATE
(26.08.98)
26 August 1998PRIORITY DATE CLAIMED
(28.08.97)
28 August 1997

TITLE OF INVENTION:

A FIBROGEN-BASED TISSUE ADHESIVE CONTAINS AN ELASTASE INHIBITOR

APPLICANT(S) FOR DO/EO/US

REDL, Heinz; SCHLAG, Günther, and EIBL, Johann

JCP

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ (X) This is the FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ () This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ (X) This express request to begin national examination procedures (35 U.S.C. 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(I).
4. ☒ (X) A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ (X) A copy of the International Application as filed (35 U.S.C. 371(c)(2)).
 - a. ☒ (X) is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☒ (X) has been transmitted by the International Bureau.
 - c. ☐ () is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ (X) A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ () Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
 - a. ☐ () are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ () has been transmitted by the International Bureau.
 - c. ☐ () have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☐ () have not been made and will not be made.
8. ☐ () A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ (X) An unexecuted oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ () A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ () An information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ () An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ (X) A FIRST preliminary amendment.
☐ () A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ () A substitute specification.
15. ☐ () A change of power of attorney and/or address letter.
16. ☒ (X) Other items of information.
 - (X) 4 Sheets of Figures as originally filed
 - (X) 4 Sheets of Figures as translated in English
 - (X) Copy of PCT Request Form.
 - (X) Copy of Cover Sheet of International Publication No. 99/11301.
 - (X) Copy of International Preliminary Examination.
 - (X) Copy of International Search Report.
 - (X) Copy of Form PCT/IB/308 (Notice Informing Applicant of the Communication of the International Application to the Designated Offices)

Certificate of Express Mail

I hereby certify that the foregoing documents are being deposited with the United States Postal Service as Express Mail, postage prepaid, "Post Office to Addressee", in an envelope addressed to the Assistant Commissioner for Patents, Box PCT, Attn: DO/EO/US, Washington, D.C. 20231 on the date indicated below.

Sean Lennon
Sean Lennon

Express Mail Label: EM355146122US
Date of Deposit: February 28, 2000

(X) Copy of Form PCT/IB/332 (Information Concerning Elected Offices Notified of Their Election).

(X) Copy of Form PCT/IB/306 (Notification of the Recording of A Change - Assignee now Baxter Aktiengesellschaft)

U.S. APPLICATION NO (if known, see 37 CFR 1.5) 09/486516		INTERNATIONAL APPLICATION NO PCT/AT98/00202		ATTORNEY'S DOCKET NUMBER BHV-317.01	
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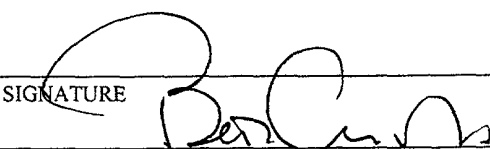
17. (x) The following fees are submitted:				CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO \$ 840.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) \$ 670.00 No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) \$ 760.00 Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$ 970.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) \$ 96.00					
ENTER APPROPRIATE BASIC FEE AMOUNT =					
Surcharge of \$130.00 for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492(e)). () 20 () 30				\$0	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	45 - 20 =	35	X \$18.00	\$630.00	
Independent claims	5 - 3 =		X \$78.00	\$156.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$260.00	\$0	
TOTAL OF ABOVE CALCULATIONS =				\$1756.00	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				\$0	
SUBTOTAL =				\$1756.00	
Processing fee of \$130.00 for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492(f)). () 20 () 30 +				\$0	
TOTAL NATIONAL FEE =				\$1756.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property. +				\$0	
TOTAL FEES ENCLOSED =				\$1756.00	
				Amount to be: refunded	\$
				charged	\$

a. (X) A check in the amount of \$1756.00 to cover the above fees is enclosed.

b. () Please charge my Deposit Account No 06-1448 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. (X) The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 06-1448, Ref. BHV-317.01. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO: Patent Group Foley, Hoag & Eliot LLP One Post Office Square Boston, MA 02109-2170	<div style="border-bottom: 1px solid black; margin-bottom: 5px;">SIGNATURE</div> <div style="text-align: center; margin-bottom: 5px;">  </div> <div style="border-bottom: 1px solid black; margin-bottom: 5px;">Beth E. Arnold</div> <div style="border-bottom: 1px solid black; margin-bottom: 5px;">REGISTRATION NO. 35,430</div>
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09/486516

430 Rec'd PCT/PTO 28 FEB 2000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:
Redl et al.

U.S. Application Serial No.: To Be Determined;
National Phase of PCT/AT98/00202
Filed 08/26/98; Priority Date: 08/28/97

For: *A Fibrogen-based Tissue Adhesive Contains
an Elastase Inhibitor*

Filed: February 28, 2000

Attorney Docket No.: BHV-317.01

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February 28, 2000
Date of Signature and of Mail Deposit

By: Sean Lennon
Sean Lennon

PRELIMINARY AMENDMENT

Dear Sir:

Please amend the above-identified patent application as follows:

In the title:

A Fibronogen-based Tissue Adhesive Contain[s]ing an Elastase Inhibitor

In the claims:

Cancel claims 1-28; and add new claims 29- 73 as follows:

WHAT IS CLAIMED IS:

---29. A tissue adhesive based on fibrinogen, said tissue adhesive comprising an admixed elastase inhibitor.

30. A tissue adhesive as set forth in claim 29, wherein said elastase inhibitor is selected from the group consisting of eglin, elastase- α 1-proteinase inhibitor, α 1-antiprotease, leukocyte protease inhibitor, elafin and mixtures thereof.

31. A tissue adhesive as set forth in claim 30, wherein said leukocyte protease inhibitor is provided as a leukocyte fraction.

32. A tissue adhesive as set forth in claim 31, wherein said leukocyte fraction is a granulocyte-derived fraction.

33. A tissue adhesive as set forth in claim 29, wherein said tissue adhesive is comprised of human proteins.

34. A tissue adhesive as set forth in claim 29, wherein said tissue adhesive is comprised of human blood proteins.

35. A tissue adhesive as set forth in claim 29, wherein said tissue adhesive is comprised of human plasma proteins.

36. A tissue adhesive as set forth in claim 29, wherein said elastase inhibitor is contained in an amount ratio of from 1:100 to 1:150,000, based on milligrams of fibrinogen.

37. A tissue adhesive as set forth in claim 29, wherein the amount of said elastase inhibitor to fibrogen in a ratio of from 1:500 to 1:110,000.

38. A tissue adhesive as set forth in claim 29, wherein said tissue adhesive contains at least 10^{-6} U of elastase inhibitor per gram of fibrinogen.

39. A tissue adhesive as set forth in claim 29, wherein said tissue adhesive contains from between 10^{-3} and 10 U of elastase inhibitor per gram of fibrinogen.

40. A tissue adhesive as set forth in claim 29, further comprising plasminogen in an amount of at least 0.0001 mg/mg of fibrinogen.

41. A tissue adhesive as set forth in claim 40, wherein said plasminogen is contained in an amount of at least 0.001 mg/mg of fibrinogen.

42. A tissue adhesive as set forth in claim 40, wherein said plasminogen is contained in an amount of more than 0.01 mg/mg of fibrinogen.

43. A tissue adhesive as set forth in claim 29, wherein said tissue adhesive does not contain any plasminogen.

44. A tissue adhesive as set forth in claim 29, further comprising at least one of a plasmin inhibitor and a plasmin activator inhibitor.

45. A tissue adhesive as set forth in claim 44, wherein said at least one of said plasmin inhibitor and said plasmin activator inhibitor is selected from the group consisting of aprotinin, α_2 -macroglobulin, α_1 -antitrypsin, ϵ -aminocaproic acid, tranexamic acid and mixtures thereof.

46. A tissue adhesive as set forth in claim 29, further comprising an antibiotic.

47. A tissue adhesive as set forth in claim 46, wherein said antibiotic is selected from the group consisting of aminoglycosides, betalactams, polypeptides, fosfomycin, tetracyclins and mixtures thereof.

48. A tissue adhesive as set forth in claim 29, further comprising factor XIII.

49. A tissue adhesive as set forth in claim 48, wherein said factor XIII is contained in an amount of at least 0.001 U/mg of fibrinogen.

50. A tissue adhesive as set forth in claim 48, wherein said factor XIII is contained in an amount of at least 0.1 U/mg of fibrinogen.

51. A tissue adhesive as set forth in claim 29, wherein said tissue adhesive is free from kininogenic proteins.

52. A tissue adhesive as set forth in claim 29, further comprising a solid surface, said tissue adhesive being present as a fleece in combination with said solid surface.

53. A tissue adhesive as set forth in claim 52, wherein said solid surface is selected from the group consisting of a collagen surface, a gelatin surface and a polysaccharide surface.

54. A tissue adhesive set forth in claim 29, wherein said tissue adhesive is resistant to lysis in an environment with high fibrinolytic activity for a period of time which is at least 10 hours.

55. A tissue adhesive as set forth in claim 29, wherein said tissue adhesive is resistant to lysis in an environment with high fibrinolytic activity for a period of time which is at least 15 hours.

56. A tissue adhesive as set forth in claim 29, wherein said tissue adhesive is lyophilized.

57. A tissue adhesive as set forth in claim 29, wherein said tissue adhesive is present in solution.

58. A tissue adhesive as set forth in claim 57, wherein said solution is deep-frozen.

59. A tissue adhesive as set forth in claim 29, wherein said tissue adhesive is present in virus-inactivated form.

60. A tissue adhesive as set forth in claim 29, wherein said elastase inhibitor is of recombinant origin.

61. A tissue adhesive system comprising fibrinogen and an elastase inhibitor.

62. A tissue adhesive system as set forth in claim 61, further comprising a thrombin-containing component.

63. A tissue adhesive system as set forth in claim 62, wherein said thrombin-containing component further comprises calcium.

64. A tissue adhesive system comprising a fibrinogen component and an elastase-inhibitor-containing component.

65. A tissue adhesive system as set forth in claim 64, wherein said elastase-inhibitor-containing component contains thrombin.

66. A tissue adhesive system as set forth in claim 61, further comprising an application device for said at least one component.

67. A tissue adhesive system as set forth in claim 66, wherein said application device is a double-syringe system.

68. A tissue adhesive system as set forth in claim 64, further comprising an application device for said fibrinogen component and for said elastase-inhibitor containing component.

69. A tissue adhesive system as set forth in claim 68, wherein said application device is a double-syringe system.

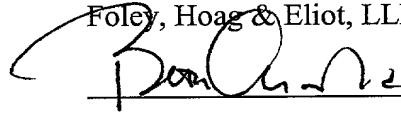
70. A method for treating wounds or hemorrhages with high fibrinolytic activity in patients, comprising administering an effective dose of a tissue adhesive preparation containing fibrinogen and an elastase inhibitor.

71. A method as set forth in claim 70, wherein said wound or hemorrhage is urological.

72. A method for treating wounds or hemorrhages in patients, comprising administering an effective dose of a tissue adhesive containing fibrinogen and an elastase inhibitor by means of an application device.

73. A method as set forth in claim 72, wherein said wound or hemorrhage is urological. ---

Respectfully submitted,
Foley, Hoag & Eliot, LLP



Date: February 28, 2000

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A Tissue Adhesive

The invention relates to a fibrinogen-based tissue adhesive.

Fibrinogen-based tissue adhesives which can also be called fibrin adhesives, in their adhesive action imitate the final phase of blood coagulation. In this instance, fibrinogen is cleaved into fibrin monomers by the action of thrombin which mostly is added to the fibrinogen solution during the glueing procedure, which, however, is also present in every wound. The fibrin monomers agglomerate spontaneously to arranged fiber-type structures called fibrin. This fibrin monomer aggregate then is further stabilized under the action of factor XIIIa by covalent cross linking. At this, in a transamidizing reaction, peptide bonds form between specific glutamine and lysine side chains of the fibrin monomers. The factor XIIIa which likewise is cleaved by thrombin from inactive factor XIII is an active transamidase and, on account of its action, it is also called "fibrin-stabilizing factor".

Although, when applying a tissue adhesive, in principle the same processes occur as in "natural" blood coagulation, in a tissue adhesive the participating components and factors are more concentrated by a multiple than in blood. Due to this, the coagulation of blood also occurs much more rapidly,

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and the achieved tissue sealing or the formed blood clot are much safer and also more stable.

A prerequisite for the breakthrough of the fibrin adhesives at the end of the 70's was the progress in the fractionation and purification of blood coagulation factors. Because of this it was possible to prepare the natural coagulation factors with such purity and at such a concentration as is required for an efficient tissue adhesion. The first commercially available tissue adhesives were launched on the market at the end of the 70's, and since then they have proven suitable in a large number of possible fields of application; primarily in those fields in which the use of conventional surgical techniques repeatedly have resulted in severe problems, e.g. with severe hemorrhages, when glueing nerves or when inner organs, such as the liver and spleen, were torn.

A further advantage of a fibrin adhesive in contrast to sutures using needle and thread resides in that the tissue or organ to be treated is not additionally damaged by the sewing procedure, and therefore, when using tissue adhesives based on fibrinogen, there are much fewer complications and less obtrusive scars than with conventional surgical sutures. Besides the optimum adhesive effect which comprises a high load bearing capacity and a high inner strength of the sealing as well as a good adherence

of the adhesive to the wound or tissue surfaces, respectively, also the processes immediately following adhesion are essential for optimizing tissue adhesives (cf. AT-B-359 652 and 359 653). Among them are the control of the durability of the sealant within the body as well as their capability of being absorbed and the adhesive's properties of enhancing wound healing.

Therefore, for a tissue adhesive not only the rapid and secure sealing effect is of decisive importance but also that the sealant formed or the blood clot formed, respectively, dissolve again in the body within a certain period of time and the wound completely heals up as a consequence of the complete absorption of the formed clot.

In this connection, it is necessary to also control the (endogenous) process of dissolving the formed blood clot, i.e. the fibrinolysis, by optimizing the tissue adhesive.

In fibrinolysis, the fibrin present in the blood clot formed is degraded and/or removed, and thereby the blood clot is dissolved. At first, under the influence of intrinsic or extrinsic plasminogen activators, such as blood coagulation factors XI and XII, prekallikrein, urokinase or t-PA, the fibrinolytically active plasmin is formed from the inactive plasminogen, said plasmin also cleaving fibrinogen and the blood coagulation

factors V and VIII in addition to fibrin.

Since the endogenous fibrinolysis processes mostly start immediately after formation of a clot and thus there is a risk that an existing tissue sealant will not adhere good enough or that a clot formed will become destabilized too early, it has become a rule in tissue glueing to provide for the addition of a plasmin inhibitor or a plasminogen activator inhibitor so as to inhibit the action of plasmin directly or indirectly, to thus protect the sealant, primarily in its initial phase, from a premature fibrinolysis. With the concentration of the inhibitor also a targeted control of the dissolution times (lysis times) of the clot formed or of the sealant, respectively, is possible. The larger the amount of inhibitor provided, the more stable the clot relative to fibrinolysis, i.e., the longer will this clot remain stable and the longer will it take for the adhesive to be completely absorbed.

Thus, when using the fibrinolysis inhibitor, an optimum balance must be found between preventing premature fibrinolysis and an as rapid as possible wound healing process.

In the commercially available tissue adhesives, aprotinin is used as the plasmin inhibitor, which is also called bovine basic pancreatic trypsin inhibitor. Aprotinin is a polyvalent proteinase (kallikrein) inhibitor and inhibits coagulation factors XIIa, XIa,

VIIIa as well as, primarily, plasmin and plasmin activators, but also trypsin, chymotrypsin and kallikrein.

Previously, aprotinin has mainly been produced from cattle. Due to the problems involved in the use of bovine material in medicaments which are used for the treatment of humans, however, more and more frequently recombinantly prepared aprotinin is being used.

In tissue sealants, aprotinin is used in an amount of from 20 to 3000 kallikrein inactivator units (KIU)/ml tissue adhesive as a rule, its optimum concentration depending on the fibrinolytic activity of the respective tissue. However, it has been shown that mainly in tissues with high fibrinolytic activity, the fibrinolysis-inhibitory action of aprotinin can be controlled to a very limited extent only, despite the use of high aprotinin concentrations, and thus undesired, early lytic processes may occur.

Thus, it is an object of the present invention to provide a tissue adhesive with which the disadvantages of the prior art are overcome and with which also primarily with tissue sealants in wounds exhibiting a high plasmin activity, a satisfactory and reliable protection against a premature fibrinolysis will be ensured, wherein the quality of the adhesion must not be negatively affected.

According to the invention, this object is achieved

by a fibrinogen-based tissue adhesive which is characterized in that it comprises an admixed elastase inhibitor. For, surprisingly, it has been shown that the fibrinolysis process cannot only be prevented by inhibiting plasmin or by inhibiting the activation of plasminogen to plasmin, but also by elastase inhibitors or by inhibitors whose fibrinolysis-inhibiting action is mainly based on a non-plasmin fibrinolysis mechanism, respectively. For reasons of simplicity, such non-plasminogen fibrinolysis inhibitors are encompassed by the term "elastase inhibitor" for the purposes of the present invention. It has been speculated that besides the plasmin-mediated fibrinolysis, also further fibrinolytic processes might exist which are not based on plasmin (e.g. a lysosomal process; cf. Simon et al., BLOOD 82 (8) (1993), pp. 2414-2422), and which cannot be substantially inhibited by aprotinin; yet, it has also been shown that this non-plasmin fibrinolytic pathway could not be inhibited by specific elastase-inhibiting peptides, such as N-methoxy-succinyl-L-alanyl-L-prolyl-L-valanyl chloromethyl ketone (AAPVCK), either (cf. Simon et al.). It was the more surprising that it could be found out in the course of the present invention that inhibitors which do not have any (substantial) plasmin- or plasminogen activator-inhibiting activities, i.e. the elastase inhibitors of the invention, can ensure a

very well controllable lytic process of the clot formed both in vitro and in vivo. This proved particularly suitable in tissues with increased fibrinolytic activity in which they can prevent early lysis even at moderate concentrations.

Premature lysis also plays a role in tissues with high fibrinolytic activity, primarily within the first time after the sealing has been made, since premature lysis may lead to a (partial) detachment of the sealant and, thus, to rebleeding.

Furthermore, it has been shown that the elastase inhibitor to be used according to the invention in the tissue adhesive exhibited its fibrinolytic activity not only in combination with conventional inhibitors acting on plasmin, but that even the entire fibrinolysis inhibition can be ensured by the elastase inhibitor alone. One particular embodiment of the present invention thus consists in that the tissue adhesive does not contain any further active components besides fibrinogen, the elastase inhibitor, and, optionally, factor XIII.

In the present adhesive, the fibrinogen concentration corresponds to that of known tissue adhesives and, as a rule, should be at least more than 50 mg, in particular more than 70-80 mg of fibrinogen/ml, i.e. at least approximately the 20-fold of the fibrinogen concentration in blood (2-4 mg/ml).

Preferably, the fibrinogen is present in a further purified form as compared to the cryoprecipitate.

Preferred elastase inhibitors within the scope of the present invention are selected from the group of eglin, elastase- α_1 -proteinase inhibitor, α_1 -antiprotease, elafin, leukocyte protease inhibitor, in particular a leukocyte fraction, preferably a granulocyte-derived fraction, or human secretory leukoprotease inhibitor, or mixtures thereof. As the leukocyte fraction, e.g., a cell lysate, in particular one derived from human cells, may be used. Other elastase- or other fibrinolysis inhibitors which do not act on plasmin may be assayed by the skilled artisan for their usefulness in the tissue adhesive of the invention in a simple manner by means of the assaying systems disclosed in the Examples or by applying the elastase inhibiting assays known from the prior art. The preferred elastase inhibitors include also various derivatives of the elastase inhibitors of the invention, e.g. fragments or forms of these inhibitors which have been modified chemically or by (recombinant) protein design, wherein, however, it is, of course, always necessary that these derivatives have the qualitative elastase-inhibitor property of the basic inhibitor.

Preferably, the tissue adhesive according to the invention is exclusively comprised of human proteins,

wherein also recombinantly prepared human proteins are to be understood as being "human proteins". Preferably, therefore, the proteins used in the tissue adhesive are prepared either from blood, plasma, cryoprecipitate, or from a recombinant cell culture.

A particularly preferred tissue adhesive is characterized in that it is exclusively composed from human blood or plasma proteins.

The ratio of the amounts of elastase inhibitor to mg of fibrinogen preferably is from 1:100 to 1:150,000, preferably from 1:500 to 1:110,000. Expressed in units of inhibitor to g of fibrinogen, preferably at least 10^{-6} U/g fibrinogen are admixed to the tissue adhesive. Particularly preferred is a range of between 10^{-3} and 10 U/g of fibrinogen. The amount of inhibitor admixed in the tissue adhesive of the invention, which inhibitor may also naturally be present in blood or plasma, respectively, preferably is at least 20x, in particular at least 50x higher than its physiological concentration in blood or plasma, respectively. The tissue adhesive of the invention may, e.g., be composed as follows: 75-115 mg/ml clottable protein, 50-110mg/ml, preferably 70-110 mg/ml, thereof being fibrinogen; optionally 1-50, preferably 10-50, IU factor XIII/ml. As the inhibitor, e.g. eglin may be admixed in an amount of between 1-100 μ g/ml or α_1 -antiprotease at 0.01-1 U/ml. As a rule it will be

sufficient to admix the elastase inhibitor in an amount which corresponds to the fibrinolysis-inhibiting activity of aprotinin in known tissue adhesives.

Depending on the purpose of the sealing, the adhesive according to the invention may contain plasminogen or may be free from plasminogen. If plasminogen is contained, it should be contained in an amount of at least 0.0001 mg/mg of fibrinogen, preferably more than 0.001, in particular more than 0.01. With the presence of plasminogen in the tissue adhesive, based on its activation to plasmin, it is also possible to define the fibrinolysis properties of the tissue adhesive even more clearly.

On the other hand, the tissue adhesive, in a further preferred embodiment, does not contain any plasminogen at all or contains only a small amount thereof, respectively.

As has been mentioned, the presence of the elastase inhibitor as the only fibrinolysis inhibitor in a tissue adhesive surprisingly suffices for the functionality of the adhesive according to the invention. Preferably, however, also a plasmin inhibitor or a plasmin activator inhibitor is used besides the elastase inhibitor, which also contributes to a better control of lysis, of absorption, and thus of wound-healing. Preferred plasmin inhibitors or plasminogen activator inhibitors are primarily

aprotinin, but also α_2 -macroglobulin, α_1 -antitrypsin, ϵ -amino-caproic acid, tranexamic acid or mixtures of these substances. Although in some instances authors have also ascribed α_1 -antitrypsin, e.g., certain actions on elastase, the substances mentioned here are viewed as plasmin or plasminogen activators, respectively, since this is the primary activity exhibited by these substances in the present field. This, of course, also applies for the purposes of the present invention. Moreover, also anti-adhesive additives, e.g. hyaluronic acid, may be contained.

A further preferred embodiment of the tissue adhesive according to the invention consists in providing an antibiotic in the adhesive, as has already been suggested in AT-B-369,990. Particularly preferred antibiotics are selected from the group of aminoglycosides, betalactams, polypeptides, phosphomycin, tetracyclines or mixtures thereof. In a further preferred embodiment, the antibiotic is present in the form of a poorly soluble derivative.

Preferably, also factor XIII is provided in the tissue adhesive according to the invention so that the inner strength of the clot and the strength and durability of the adhesion will be positively influenced. To this end, factor XIII is preferably used in an amount of from 1-50 units/ml, preferably around 10 U/ml. Based on fibrinogen, factor XIII is preferably

present in a minimum concentration of 0.001 U/mg of fibrinogen, in particular at least 0.1 U/mg of fibrinogen. Depending on the type of adhesion or type of tissue, however, the optimum factor XIII concentration may easily be optimized by any skilled artisan. If an antibiotic is present in the tissue adhesive, it is basically recommendable to provide somewhat more factor XIII (cf. AT-B-369,990).

Preferably, the tissue adhesive according to the invention is free of kininogenic proteins (such as, e.g., kallikrein, etc.), whereby interfering side reactions can be prevented from the beginning.

According to a preferred embodiment, the tissue adhesive according to the invention is present in combination with a solid surface as a fleece, whereby primarily for large-area wounds, an optimum wound closure and an optimum covering will be attained. Examples of such fleeces have been listed in AT-B 374,367. The solid surface of the fleece thus preferably is a collagen, gelatin or polysaccharide surface, wherein, of course, also further medically suitable surfaces which optionally may also have been impregnated for the specific purpose of use may be employed.

It has been shown that according to the invention, with the tissue adhesive comprising an elastase inhibitor a resistance to lysis can be achieved for a

period of at least 10 hours, preferably 15 hours, even in an environment with high fibrinolytic activity. Resistance to lysis according to the present invention means that a respective fibrin clot will not be degraded within a certain period of time, i.e. it remains in existence. Determination of the resistance to lysis is, e.g., effected by a photometric measurement in dependence on time. A preferred tissue adhesive according to the present invention thus has a resistance to lysis of at least 10 hours, preferably at least 15 hours, in an environment of high fibrinolytic activity. By "high fibrinolytic activity", e.g., a plasmin activity is understood which is above the physiological plasmin potential. The fibrinolytic potential may, e.g., be expressed by the plasminogen concentration (cf., e.g., Henriksson et al., Thrombosis Research 16: 301-312; 1979). This property of a resistance to lysis may be checked by any skilled artisan by a simple assay, as described in the Examples.

When being applied, the tissue adhesive of the invention preferably is present in solution, yet for storage purposes, either deep freezing of the solution so that the tissue adhesive is present in deep-frozen form, or lyophilizing of the adhesive, i.e. providing it in lyophilized form, is recommendable. By "lyophilized form", of course, only a tissue adhesive

preparation made storable by freeze-drying is to be understood which in a subsequent reconstitution thereof can be reconstituted almost completely (i.e. to at least 80%) within a few minutes at 37°C.

The tissue adhesive according to the invention advantageously is present in virus-inactivated form.

This inactivation treatment preferably is ensured by a tenside and/or heat treatment, e.g. by a heat treatment in the solid state, in particular by a vapour treatment according to EP-0 159 311, or EP-0 519 901 or EP-0 674 531.

Further treatments for inactivation of viruses also comprise a treatment by chemical or chemical/physical methods, e.g. with chaotropic substances according to WO94/13329, DE 44 34 538 or EP 0 131 740 (solvent), or photoinactivation.

Nanofiltration also is a preferred method for depleting viruses within the scope of the present invention.

According to a preferred embodiment, the elastase inhibitors admixed according to the invention may also be of recombinant origin.

Furthermore, the present invention relates to a tissue adhesive system comprising, as one component thereof, a tissue adhesive according to the invention which comprises an elastase inhibitor.

As a rule, the tissue adhesive system of the

invention comprises a thrombin component as a further component, in which thrombin is present either in liquid form or as a lyophilisate capable of being reconstituted, wherein the thrombin component may have different concentrations when used in the adhesion, depending on the field of application.

A tissue adhesive system which also falls within the scope of the present invention is characterized in that it comprises a fibrinogen component and a component separate therefrom which contains an elastase inhibitor. As a rule, however, it is suitable to provide the fibrinolysis inhibitor component in the fibrinogen component (cf. AT-B-359,652 and 359,653). By suitable application devices, the inhibitor component may, however, also be supplied separately from the fibrinogen component. Preferably, that component which contains an elastase inhibitor at the same time also contains thrombin, it again being possible to provide this component either as a lyophilisate or as an (optionally deep-frozen) solution.

The tissue adhesive systems according to the invention further comprise suitable application devices for the system component(s). In particular, double syringe systems as described in EP 0 037 393, EP 0 210 160 or EP 0 292 472, or application devices as described in EP 0 315 322 or EP 0 669 100 have proven suitable. With these special application devices, also

those embodiments in which the inhibitor is applied in the thrombin component will yield reliable adhesive results.

The present adhesive is suitable for all the fields of known applications possible for fibrin adhesives. It has, however, proven particularly suitable when providing adhesions in fields with high fibrinolytic activity. Thus, a subject matter of the present invention is also the use of the tissue adhesive of the invention or of a tissue adhesive system of the invention, respectively, for producing a preparation or an application device, respectively, to be used in fields with high fibrinolytic activity, in particular in urology. A subject matter of the present invention is, moreover, a method for using a tissue adhesive of the invention or a tissue adhesive system of the invention, respectively, in surgery in fields involving high fibrinolytic activity, in particular in urology.

The invention will now be explained in more detail by way of the following Examples and drawing figures, to which, however, it shall not be restricted.

Fig. 1 shows the decrease of extinction corresponding to an increasing lysis of the clot

- a) fibrin adhesive without aprotinin
- b) fibrin adhesive with aprotinin (1,000 U/ml)
- c) fibrin adhesive with alpha-1 PI (0.01 U/ml)
- d) fibrin adhesive with alpha-1 PI (0.001 U/ml)

e) fibrin adhesive with alpha-1 PI (0.0001 U/ml);

Fig. 2 shows the decrease of extinction corresponding to an increasing lysis of the clot

a) fibrin adhesive with aprotinin (1,000 U/ml)

b) fibrin adhesive with eglin (1 µg/ml)

c) fibrin adhesive without aprotinin;

Fig. 3 shows the extent of rebleeding, expressed by the increase in the weight of the pre-weighed pads, in a hyper-fibrinolytic environment, which had been induced by infusion of t-PA; and

Fig. 4 shows the extent of rebleeding, expressed by the increase in the weight of pre-weighed pads, in an environment with normal fibrinolytic activity, i.e. without t-PA infusion.

Examples:

1. In vitro-tests for assaying the fibrinolysis-inhibiting action of the tissue adhesive according to the invention (at present considered by applicant to be the best mode of carrying out the invention)

In this Example, blocking of the lysis of a tissue adhesive clot by means of eglin or α_1 -antiprotease is shown. In this instance, the tissue adhesive STIM3 (IMMUNO AG, Vienna, AT) (comprising 70 mg of fibrinogen/ml) is dissolved in water and subsequently diluted 1:6 with an 0.9 M NaCl solution.

This tissue adhesive solution is mixed 1:1 with a thrombin solution that had been dissolved in 40 mM

CaCl₂ and subsequently had been diluted with a 40 mM CaCl₂/0.9 M NaCl (1:5) solution to 0.1 U/ml, and pipetted on a micro plate, with 100 µl/well being provided.

Various concentrations of the inhibitors were added to each 5 µl of tissue adhesive (Eglin 1-100 µg/ml, α₁-antiprotease 0.01-1 U/ml).

For hardening of the adhesive, the microtiter plate was incubated at 37°C for approximately 1.5 hours. The corresponding lysis reagents (a: cell-free supernatant from leukocyte homogenate (3x freezing/thawing) of 500,000 leukocytes/µl; b: t-PA 2 mg/ml as positive control; NaCl 0.9% as negative control) were then pipetted onto the clots (100 µl/well). Subsequently, the microtiter wells were measured in the plate photometer at 37°C at a wave length of 405 nm kinetically over night 60x900 s in the Photometer SLT 340 ATTC. The results are illustrated in Figs. 1 and 2, the decrease in the extinction corresponding to the increasing lysis of the clots.

It has been shown that both, with ≥ 1 µg of eglin/ml and with ≥ 0.01 U of α₁-antiprotease/ml it is possible to prevent the lysis of the fibrin clot which occurs in the assay within 15 hours, which, on the one hand, is a hint to the central role played by the leukocyte proteases for the degradation of the fibrin clot and, on the other hand, shows the excellent effect

of the inventive elastase inhibitors for preventing this lysis.

2. In vivo activity of the elastase inhibitors used according to the invention

To determine the importance of leukocyte proteases, in particular elastase inhibitors, within the scope of the present invention, the effect of the tissue adhesive of the invention both in hyper-fibrinolytic systems and in case of normal fibrinolytic activity were tested to illustrate hemostasis by means of tissue adhesive and compared with adhesives without inhibitors, and with an adhesive containing only aprotinin as plasmin inhibitor, respectively.

2.a) Hyperfibrinolysis

Anesthetized rabbits (2-3 kg) were heparinized (4,000 U/kg). Half an hour later, a part of the right liver lobe was clamped and partially resected distal from the clamp. Hemorrhages from larger vessels were stopped by electrocoagulation, and the residual diffuse haemorrhage was sealed by applying tissue adhesive (max. 4 ml) within 200 seconds. 10 minutes later, infusion of t-PA (700 U/kg/h) was started, and the extent of rebleeding was determined for 2 hours by measuring the increase in weight of pre-weighed pads.

In doing so, 3 different tissue adhesives were tested.

a) Tissue adhesive (STIM3) with aprotinin

(3,000 U/ml) as negative control

b) Tissue adhesive (STIM3) without aprotinin as positive control

c) Tissue adhesive (STIM3) without aprotinin, with eglin (10 µg/ml); adhesive according to the invention

These adhesives were blind-tested and applied by means of a Duploject® syringe (from IMMUNO, Vienna, AT).

The results obtained are illustrated in Fig. 3.

2.b) Normal fibrinolytic activity

In addition to the hyperfibrinolysis model, also the same assays were carried out without t-PA infusion, yet with increased observation periods of 4 hours. The results obtained are illustrated in Fig. 4.

It has been shown that both, in the hyperfibrinolysis model and with normal fibrinolysis, reduced rebleeding is achieved as compared to conventional adhesives, with improved properties as compared to aprotinin, primarily in case of longer lysis periods.

These results prove the excellent effects of the tissue adhesives according to the invention, with which an early lysis of the fibrin adhesive can be prevented, whereby rebleedings could be prevented also in fields with high fibrinolytic activity.

Claims:

1. A fibrinogen-based tissue adhesive, characterized in that it comprises an admixed elastase inhibitor.
2. A tissue adhesive according to claim 1, characterized in that the elastase inhibitor is selected from the group of eglin, elastase- α_1 -proteinase inhibitor, α_1 -antiprotease, leukocyte protease inhibitor, elafin, or mixtures thereof.
3. A tissue adhesive according to claim 2, characterized in that the leukocyte protease inhibitor is provided as a leukocyte fraction, in particular a granulocyte-derived fraction.
4. A tissue adhesive according to any one of claims 1 to 3, characterized in that it is composed exclusively of human proteins.
5. A tissue adhesive according to any one of claims 1 to 4, characterized in that it is composed exclusively of human blood- or plasma proteins.
6. A tissue adhesive according to any one of claims 1 to 5, characterized in that the elastase inhibitor is contained in an amount ratio of from 1:100 to

1:150,000, preferably 1:500 to 1:110,000, based on mg of fibrinogen.

7. A tissue adhesive according to any one of claims 1 to 6, characterized in that at least 10^{-6} U of elastase inhibitor are contained per g of fibrinogen, preferably between 10^{-3} and 10 U/g of fibrinogen.

8. A tissue adhesive according to any one of claims 1 to 7, characterized in that it contains plasminogen in an amount of at least 0.0001 mg/mg of fibrinogen, preferably at least 0.001, most preferred more than 0.01.

9. A tissue adhesive according to any one of claims 1 to 7, characterized in that it does not contain any plasminogen.

10. A tissue adhesive according to any one of claims 1 to 9, characterized in that it further comprises a plasmin inhibitor or a plasmin activator inhibitor which preferably is selected from the group of aprotinin, α_2 -macroglobulin, α_1 -antitrypsin, ϵ -aminocaproic acid, tranexamic acid, or mixtures thereof.

11. A tissue adhesive according to any one of claims

1 to 10, characterized in that it comprises an antibiotic which preferably is selected from the group of aminoglycosides, betalactams, polypeptides, phosphomycin, tetracyclines or mixtures thereof.

12. A tissue adhesive according to any one of claims 1 to 11, characterized in that it comprises factor XIII, preferably in an amount of at least 0.001 U/mg of fibrinogen, particularly preferred at least 0.1 U/mg.

13. A tissue adhesive according to any one of claims 1 to 12, characterized in that it is free from kininogenic proteins.

14. A tissue adhesive according to any one of claims 1 to 13, characterized in that it is present in combination with a solid surface as a fleece.

15. A tissue adhesive according to claim 14, characterized in that the solid surface is a collagen, gelatin or polysaccharide surface.

16. A tissue adhesive according to any one of claims 1 to 15, characterized in that in an environment of high fibrinolytic activity it is resistant to lysis for a period of time of at least 10 hours, preferably at least 15 hours.

17. A tissue adhesive according to any one of claims 1 to 16, characterized in that it is lyophilized.

18. A tissue adhesive according to any one of claims 1 to 16, characterized in that it is present in solution.

19. A tissue adhesive according to claim 18, characterized in that the solution is deep-frozen.

20. A tissue adhesive according to any one of claims 1 to 19, characterized in that it is present in virus-inactivated form.

21. A tissue adhesive according to any one of claims 1 to 20, characterized in that the elastase inhibitor is of recombinant origin.

22. A tissue adhesive system, characterized in that it comprises a tissue adhesive according to any one of claims 1 to 21 as one component thereof.

23. A tissue adhesive system according to claim 22, characterized in that it further comprises a component which comprises thrombin and, optionally, calcium.

24. A tissue adhesive system, characterized in that it comprises a fibrinogen component and a component which comprises an elastase inhibitor.

25. A tissue adhesive system according to claim 24, characterized in that the component which comprises an elastase inhibitor comprises thrombin.

26. A tissue adhesive system according to any one of claims 22 to 25, characterized in that it further comprises an application device for the component(s) of the system, in particular a double-syringe system.

27. The use of a tissue adhesive according to any one of claims 1 to 19 for producing a preparation to be applied in fields with high fibrinolytic activity, in particular in urology.

28. The use of a tissue adhesive sytem according to any one of claims 22 to 26 for producing an application device to be employed in fields with high fibrinolytic activity, in particular in urology.

A Tissue Adhesive

A b s t r a c t :

A fibrinogen-based tissue adhesive is disclosed which comprises an elastase-inhibitor.

(Fig. 1)

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1/4

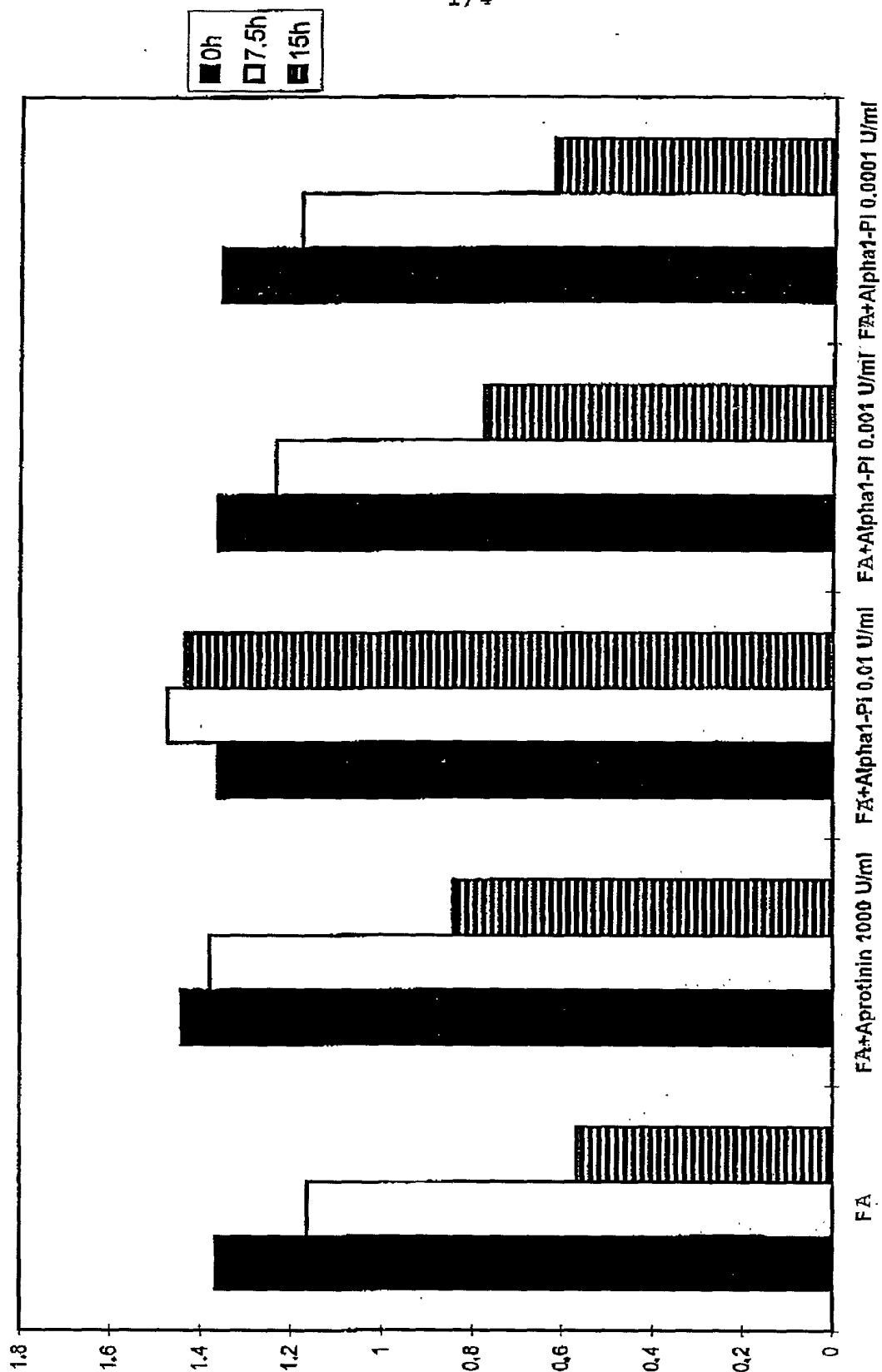


Fig.1

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2/4

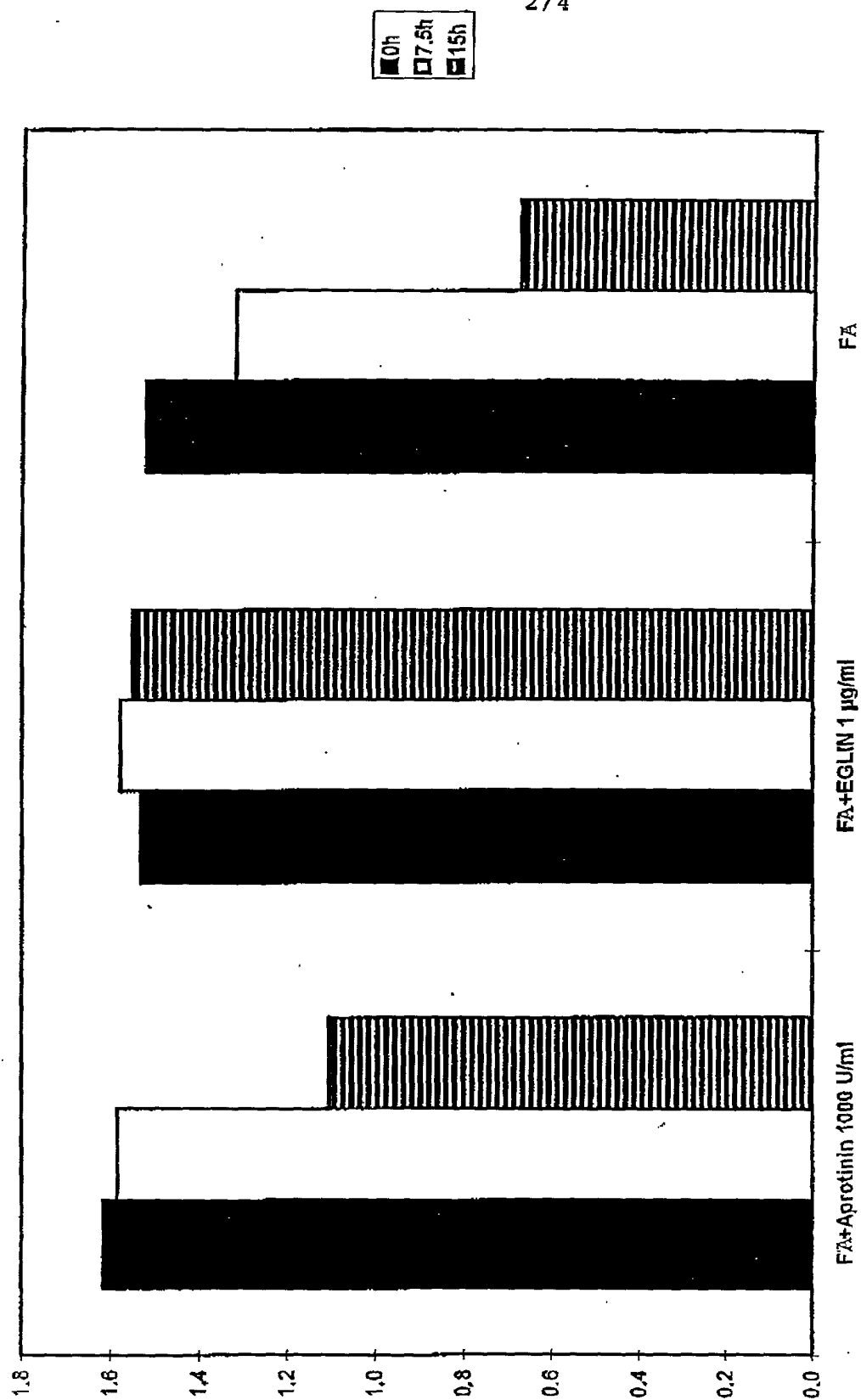


Fig. 2

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Blood loss after partial liver lobe resection after use of
FA with/without eglin (phase 3, t-PA)

(mean values \pm STD)

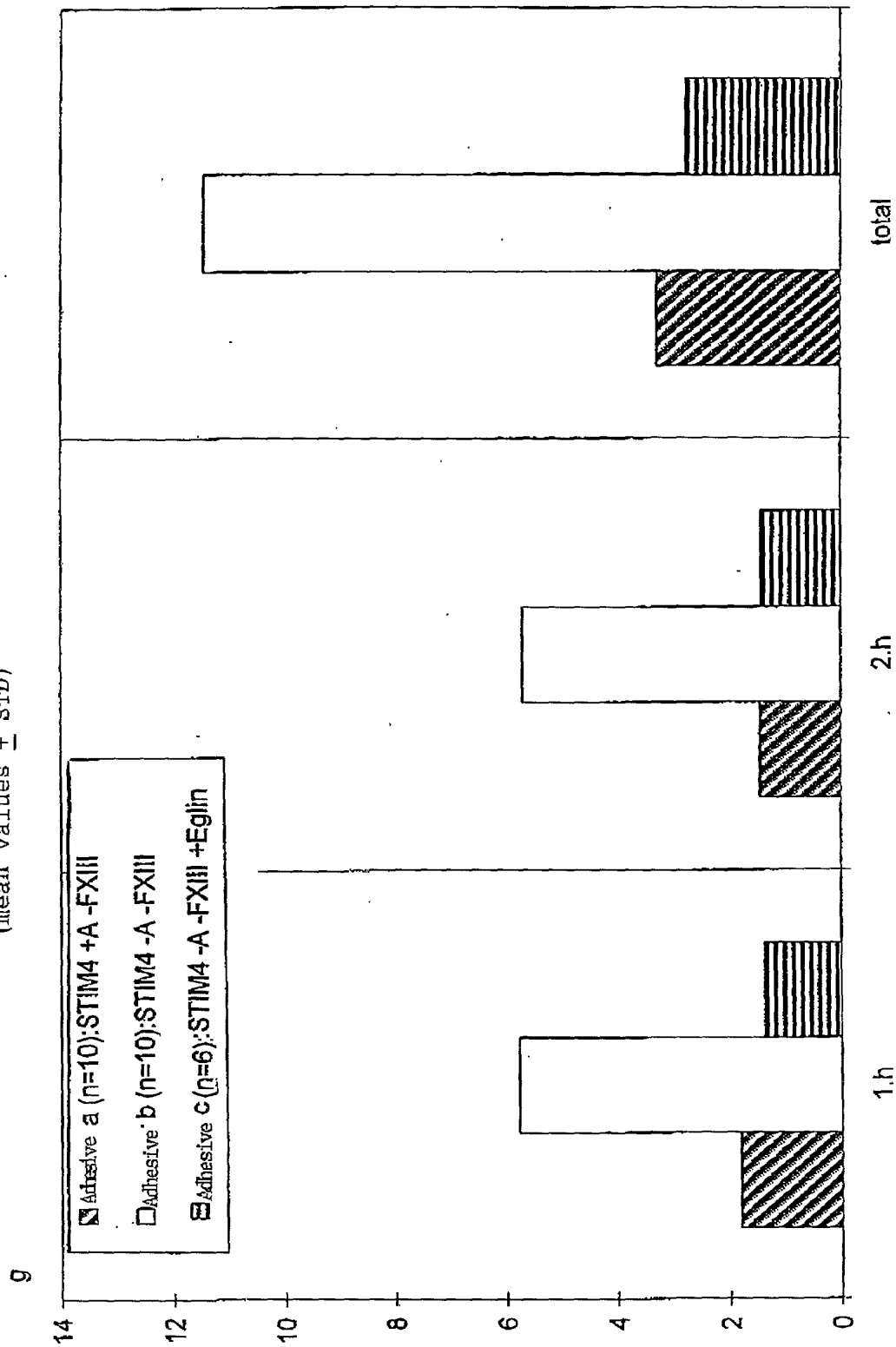


Fig.3

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Blood loss after partial liver lobe resection after use of

FA with/without eglin

(mean value \pm STD)

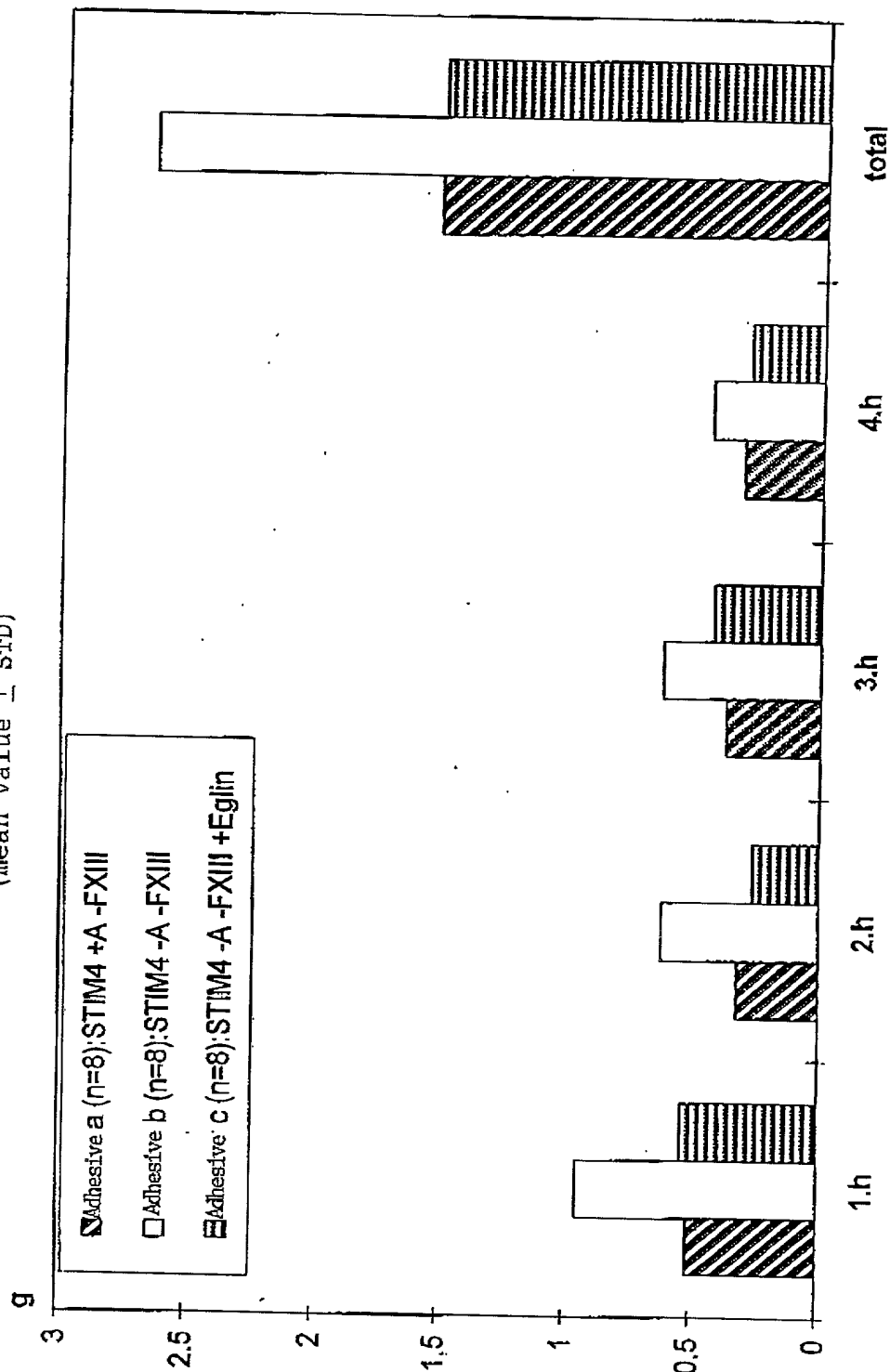


Fig.4

07 JUN 2000

DECLARATION FOR PATENT APPLICATION

Docket Number: _____

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

FIBRINOGEN-BASED TISSUE ADHESIVE

the specification of which (check one): ☐ is attached hereto, 1998 International Patent
☒ was filed on 08/26/ as United States Application Number
 PCT/AT98/00202, and was amended on _____ (if
 applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulation, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Priority Claimed

<u>A 1449/97</u> (Number)	<u>Austria</u> (Country)	<u>28/August/1997</u> (Day/Month/Year Filed)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	<input type="checkbox"/> Yes <input type="checkbox"/> No

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States Provisional application(s) listed below.

(Application Number) _____
(Filing Date)

(Application Number) _____
(Filing Date)

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

(Application Number) _____
(Filing Date) _____
(Status: patent, pending, abandoned)

(Application Number) _____
(Filing Date) _____
(Status: patent, pending, abandoned)

9 I hereby appoint Beth E. Arnold, Reg. No. 35,430; Paula Campbell, Reg. No. 32,503; Charles H. Cella, Reg. No. 38,099; Isabelle M. Clauss, Reg. (*see attached*); Edward J. Kelly, Reg. No. 38,936; Donald W. Muirhead, Reg. No. 33,978; Chinh Pham, Reg. No. 39,329; Diana Steel, Reg. No. 43,153; Matthew P. Vincent, Reg. No. 36,709; and Anita Varma, Reg. No. 43,221 as attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

Address all telephone calls to Beth E. Arnold at telephone number (617) 832-1000.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor (given name, family name): Heinz REDL

Inventor's signature _____ Date _____
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Residence _____ Citizenship _____
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~~Inventor's signature~~ *Irmgard Schlag* _____ Date 15.03.2000
of Guenther Schlag _____
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Post Office Address (if different) _____

07 JUN 2000

DECLARATION FOR PATENT APPLICATION

Docket Number: BHV-317.01

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

09/15/98

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

FIBRINOGEN-BASED TISSUE ADHESIVE

the specification of which (check one): () is attached hereto. International Patent
(X) was filed on 08/26/1998 as United States Application Number
PCT/AT98/00202, and was amended on _____ (if
applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

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<u>A 1449/97</u> (Number)	<u>Austria</u> (Country)	<u>28/August/1997</u> (Day/Month/Year Filed) (X) Yes () No
<u>PCT/AT98/00202</u> (Number)	<u>PCT</u> (Country)	<u>26/August/1998</u> (Day/Month/Year Filed) (X) Yes () No

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(Application Number) (Filing Date)

(Application Number) (Filing Date)

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(Application Number) (Filing Date) (Status: patent, pending, abandoned)

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Full name of sole or first inventor (given name, family name): Heinz REDL

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18/February/2000

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Full name of third inventor (given name, family name): Johann EIBL

Inventor's signature

18/February/2000

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